

NOTES ON THE BIOLOGY OF *DIGLYPHUS INTERMEDIUS*
(HYMENOPTERA: EULOPHIDAE), A PARASITE OF THE
ALFALFA BLOTCH LEAFMINER, *AGROMYZA FRONTELLA*
(DIPTERA: AGROMYZIDAE)

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Abstract.—*Diglyphus intermedius* (Girault) is the most common parasite of the alfalfa blotch leafminer, *Agromyza frontella* (Rondani), on alfalfa in Delaware, southcentral Pennsylvania, and southern New Jersey. It is a larval ectoparasite of leafminer larvae and is usually solitary; however, occasionally 2–5 parasites develop on a single host. It paralyzed and killed all three larval instars of its host agromyzids without ovipositing though it usually oviposited and developed on 3rd-instar larvae. The parasite has three instars as measured by changes in mandible length. At a constant 25.5°C, it developed from egg to adult in 11 days, lived 3–4 weeks as an adult and had an average fecundity of 40 offspring per female.

The alfalfa blotch leafminer (ABL), *Agromyza frontella* (Rondani), an introduced pest of European origin, was first observed in the USA in Hampshire County, Massachusetts, in 1968 (Miller and Jensen, 1970). It is usually a subeconomic pest on alfalfa throughout the northeastern USA and adjoining Canadian provinces, but occasionally it has become a serious pest (100–200 adults collected/sweep (Kim, 1975)). We report here notes on the biology of the most important of the native parasites of ABL, *Diglyphus intermedius* (Girault).

Diglyphus intermedius is a Nearctic species that has been reported to parasitize *Liriomyza subpusilla* Frost in California and *Phytomyza atricornis* Meigen in Rhode Island (Peck, 1963); *Liriomyza sativae* Blanchard (= *Liriomyza prosperpusilla* Frost) in Florida and Illinois (Burks, 1967); *Phytomyza chrysanthemi* Kowarz in Rhode Island (Peck, 1951); and *Liriomyza brassicae* (Riley) in California (Spencer, 1973). We have collected it from *Liriomyza trifoliarum* Spencer on alfalfa throughout the northeastern USA.

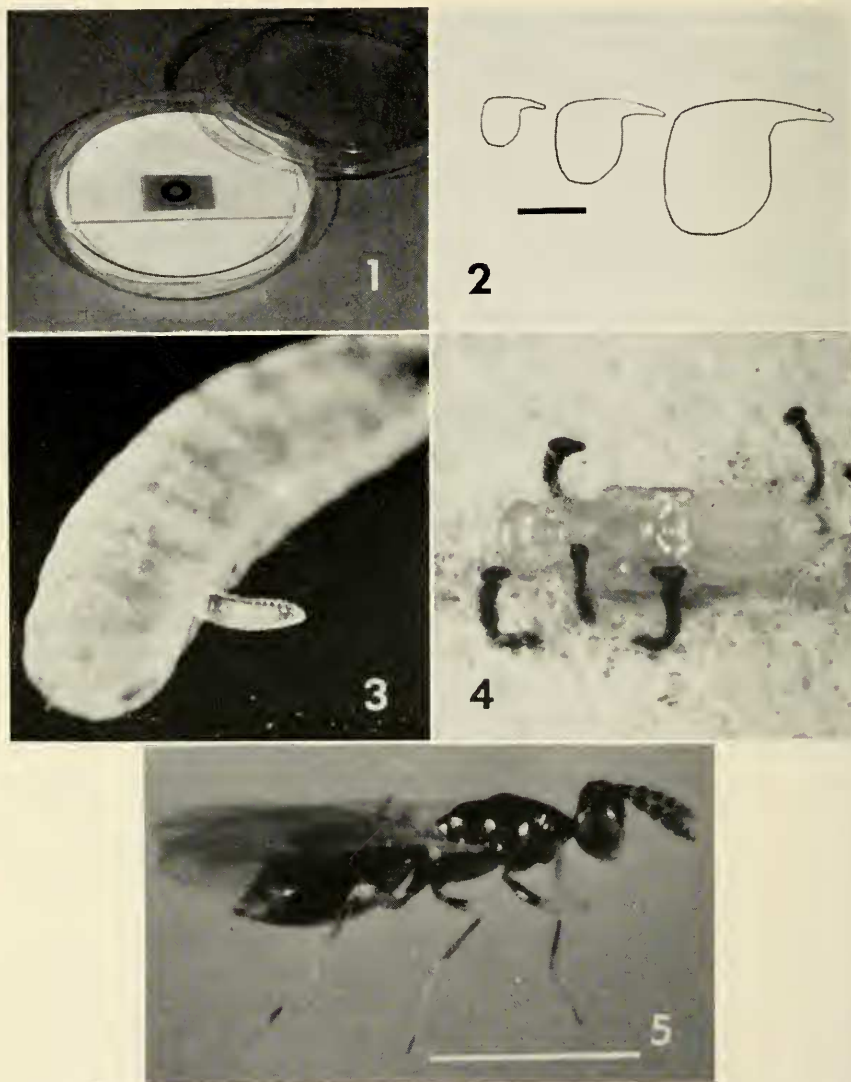
Laboratory Studies

All the life history studies of *Diglyphus intermedius* in the laboratory were conducted on a native agromyzid, *Liriomyza trifoliarum* Spencer, obtained from field-collected alfalfa and maintained in a laboratory colony on "Bountiful" bush snap bean, *Phaseolus vulgaris* L., and "Thaxter" lima bean, *Phaseolus limensis* Macf., in caged plants at $25.5 \pm 1.1^\circ\text{C}$ and $60 \pm 5\%$ RH with a photoperiod of 16L:8D. Observations on larval development

were made by constructing artificial mines because *Diglyphus intermedius* develops entirely within the host's mine, and development there is obscured by the leaf tissue. The mine consisted of a microscope slide on which we placed a small square of blotter paper. A circle of construction paper with a hole in it, similar in size and shape to the gummed reinforcements used for notebook paper, was laid on the blotter paper. In the central hole was placed a parasitized leafminer larva that had been dissected from the leaf mine. A coverslip was placed on top of the larva to prevent any mobile parasite larva from leaving the artificial mine. The entire slide was placed in a closed petri dish that had moistened plaster of Paris (Fig. 1) or sand on the bottom to maintain a high RH. The slide was removed from the petri dish for observation by microscope. Since the majority of parasites handled in this way survived from egg to adult, the technique could be applied to the study of other larval ectoparasites of leafminers.

The number of instars was determined by exposing 3rd-instar hosts to adult parasite adults for 15 h. When the parasites were removed at 0800 h, that hour was counted as the beginning of the first day of maturation of the parasite though, in some cases, as much as 15 h had elapsed. Then at 0800 h on succeeding days, parasite larvae were removed and mounted on slides in Hoyer's medium. The instar was determined by measuring the length of the mandibles from the tip of the "tooth" to the posterior margin of the dorsal process (the greatest distance across the mandible). The length of a single mandible averaged $10\ \mu$ ($n = 12$, range = $6\text{--}13\ \mu$) for the 1st instar, $17\ \mu$ ($n = 7$, range = $15\text{--}19\ \mu$) for the 2nd instar, and $27\ \mu$ ($n = 11$, range = $23\text{--}30\ \mu$) for the 3rd instar (Fig. 2). These observations were corroborated by measuring the mandibles of larvae in eggs shortly before eclosion, and those in cast skins. Therefore, we concluded that the parasite had three larval instars. A 1st-instar parasite larva feeding on a *Liriomyza trifoliarum* larva is shown in Fig. 3.

From the observations of the larval parasites in artificial mines, it was found that at 25.5°C development from egg to adult required 11 days: 1 day for the egg stage, 4 for the larval stage and 6 for the pupal stage. The egg hatched within 24 h after it was laid, and if it was not already attached to the host, the young parasite larva sought out the host and began feeding immediately. The 1st instar lasted about 24 h, the 2nd less than 24 h and the 3rd about $2\frac{1}{2}$ days. On the 3rd day, the larva changed from light yellow to a lime-bluish green and soon constructed 6–8 meconial pillars that extended from the upper to the lower surface of the mine. Since these pillars are usually arranged in pairs on each side of the pupa (Fig. 4), they apparently protect the pupa from being crushed if the leaf dries out and also prevent the pupa from rolling about inside the mine. When the parasite transformed to the adult stage (Fig. 5) in the host's mine, it chewed an oval hole in the upper epidermis of the leaf and left the mine.



Figs. 1-5. 1. Artificial mine. Parasite and host are inside black paper ring with coverslip placed on top. Plaster of Paris in petri dish was moistened to maintain high humidity; 2. Mandibles of 1st-, 2nd-, and 3rd (final)-instar *Diglyphus intermedius* larvae. Bar is 10 μ ; 3. First-instar *D. intermedius* larva feeding on 3rd-instar *Liriomyza trifoliarum* host. Bar is 0.1 mm; 4. Meconial pillars around *Diglyphus intermedius* pupa. Tops of pillars are attached to underside of coverslip; 5. Adult female *D. intermedius*. Bar is 1 mm.

Repeated attempts to observe mating in cages of various sizes, with and without hosts, were unsuccessful. Mating was infrequently observed in maintenance culture cages containing thousands of parasites.

Oviposition of *Diglyphus intermedius* was studied by confining parasite adults in a petri dish with a bean leaf mined by 3rd (final)-instar hosts. The activity could then be observed through a microscope. The searching behavior of the female parasite consisted of palpating the leaf until a mine was located. Then she followed the mine by swaying back and forth, almost in a rhythmic motion, while she palpated the mine alternately with each antenna. Once she discovered a host, she immediately circled above it and palpated the leaf epidermis with her antennae. The host larva apparently sensed the presence of the parasite above the mine and often began to thrash around within the confines of the mine, even before the parasite penetrated the mine with the ovipositor. Within seconds after locating a host, the parasite attempted to sting it and the host responded to each attempted sting by making a rapid twisting motion in an apparent attempt to avoid the ovipositor. The parasite was observed to sting and kill each of the three host instars, but oviposition was never observed on 1st-instar larvae. Stinging without oviposition lasted from 2–14 s. After several stings, the host ceased movement, but the hindgut was observed to continue to function until waste matter was expelled; then the larva became completely immobile. More prolonged stings, average 39 s ($n = 6$, range = 25–53 s), were assumed to be oviposition attempts. The parasite commonly oviposited 1–2 eggs/host, but on one occasion three eggs were laid. Eggs were ordinarily placed on the host larva, but occasionally some were placed 1–2 mm from the host but within the mine. Oviposition usually occurred on 3rd-instar hosts, but in one case, a female was observed to sting a 2nd-instar larva nine times and to lay one egg on the host. Since attempts to rear the parasite on 2nd-instar host larvae were unsuccessful, we suspect that such *Liriomyza trifoliarum* larvae do not usually provide sufficient food to allow complete development of the parasite.

Fecundity of the parasite was determined by confining a single female, less than 24 h old, with two males on "Bountiful" bush snap beans heavily infested with 3rd-instar *L. trifoliarum*. The parasites were supplied with honey as food, and fresh host material was placed in the cage weekly until the female died. The plant material was removed and held for parasite emergence. The average fecundity was found to be 40.2 progeny per female ($n = 6$). Females in the test lived 3–4 weeks.

Parasites reared on "Bountiful" bush snap bean had a sex ratio of 1 ♀:2.2 ♂ ($n = 204$). However, the ratio on "Thaxter" lima bean was 1 ♀:1 ♂ ($n = 117$). A similar disparity was noted when we reared the European parasite *Diglyphus isaea* (Walker) on these two bean species.

Field Observations

Samples of 50 alfalfa leaflets mined by either ABL or *Liriomyza* spp. were collected weekly from early May through November 1975, from each of seven fields (one at Newark, Delaware; three near Oxford, Pennsylvania; and three near Rancocas, New Jersey) unless the alfalfa was cut or bad weather prevented fieldwork. If populations of agromyzid larvae were low, a sample consisted of as many mined alfalfa leaflets as could be collected in each field in 20 min. In the laboratory, the mined leaflets were placed in petri dishes (50 mm diam with tight-fitting lids) on slightly moistened filter paper, which retained moisture for 3–4 weeks, long enough for the parasites to develop and emerge at $22.2 \pm 1.1^{\circ}\text{C}$. The host larvae were separated by mine character. ABL, in the 1st and early 2nd instar, formed a fairly straight linear mine; in late 2nd and throughout the 3rd instar it produced a large blotch mine. *Liriomyza* spp. formed a serpentine, linear mine in all three instars. The instar of the host was determined by measuring the length of the cephalopharyngeal skeleton by transmitted light under the microscope and comparing it to predetermined length ranges for each instar for each species.

Diglyphus intermedius parasitized a total of 9.2% of the ABL larvae collected from the field ($n = 4,748$), but actual field mortality due to the parasite was probably higher than that. We did not include larvae killed by parasite stinging without oviposition because they could not be easily separated from those killed by predators, diseases, overcrowding in the leaflet, or environmental factors. Total parasitism of ABL by all parasitic species was 18.8% and *D. intermedius* accounted for almost 50% of this amount. *Diglyphus intermedius* preferred 3rd (final)-instar larvae, although 23.7% of the hosts were 2nd-instar larvae. Thus our inability to rear the parasite on 2nd-instar *Liriomyza trifoliarum* larvae in the laboratory was probably caused by the smaller size of the host.

The parasite was usually solitary but was gregarious on 13.3% of the larvae (2–5 adults from a single host).

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